Clin. Med., 134(3):215-221 (1999)). Here as well, a treatment that would allow the protein to exit the ER might restore anti-bacterial phagocytic function to individuals suffering from MPO deficiency.

Congenital Insulin Resistance. The hormone binding site of the insulin receptor is contained in the extracellular region of the protein. In this form of type A insulin resistance, substitution mutations of residues located in the beta-sheet and at the hormone-binding region completely disrupt intracellular folding and movement of the protein, resulting in aberrant retention at an incorrect cellular location.

Misfolded receptors remain bound to calnexin molecules in the endoplasmic reticulum until they are degraded. As previously discussed in connection with other diseases, a treatment providing release and cellular export of the mutant receptor could have wide-spread therapeutic use.

Nephrogenic Diabetes Insipidus. Nephrogenic diabetes insipidus is characterized by an inability to concentrate urine in spite of normal or increased plasma concentrations of the antidiuretic hormone arginine vasopression (AVP), which normally stimulates water reabsorbtion in the distal tubules and/or collecting ducts of the kidney by regulating the expression of "water channels" known as aquaporins. In the collecting duct, binding of AVP to the vasopressin 2 receptor triggers a cascade -- activation of the receptor-linked G protein G_s, activation of adenylate cyclase, and stimulation of protein kinase A, eventually leading to exocytic insertion of specific water channels, aquaporin 2, into the luminal membrane of collecting duct cells. Presence of these channels increases permeability of the luminal membrane. Thus short term regulation of AQP2 by AVP entails movement of AQP2 from intracellular vesicles to the plasma membrane. Longer term regulation occurs through increased abundance of APQ2, which is thought to result from increased transcription of the *AQP2*gene. AVP also increases renal water reabsorption through a variety of additional mechanisms. Nephrogenic diabetes insipidus is comprehensively reviewed in Morello, J. and Bichet, D., Nephrogenic diabetes insipidus, *Annu. Rev. Physiol.*, 63:607-30, 2001.

Nephrogenic diabetes insipidus can be inherited or acquired. Polyuria and polydipsia are the major symptoms. Approximately 90% of patients with congenital nephrogenic diabetes insipidus have an X-linked form of the disorder caused by mutations in the arginine vasopressin receptor 2 gene (*AVPR2*). In less than 10% of families studied the disorder has an autosomal recessive or autosomal dominant pattern of inheritance. Mutations in the aquaporin-2 gene (*AQP2*) have been identified in some

of these kindreds. Based on studies of glycosylation patterns, it is apparent that most *AVPR2* mutations lead to receptors that are trapped in a pre-Golgi compartment, presumably the ER, and are thus unable to reach the cell surface (See Morello and Bichet, 2001 and papers referenced therein). AQP-2 mutations that cause autosomal recessive nephrogenic diabetes insipidus are also characterized by misfolded mutant proteins that are trapped in the ER (Kamsteeg, E.J., *et al.*, An impaired routing of wild-type aquaporin-2 after tetramerization with an aquaporin-2 mutant explains dominant nephrogenic diabetes insipidus; reviewed in van Os, C.H. and Deen, P.M., Aquaporin-2 water channel mutations causing nephrogenic diabetes insipidus, *Proc. Assoc. Am. Physicians*, 110(5): 395-400, 1998). Thus agents and methods such as those described herein, that allow release of misfolded proteins from the ER, are likely to be useful in the treatment of congenital nephrogenic diabetes.

Hereditary Hemochromatosis. Hemochromatosis is a common autosomal recessive disorder characterized by excessive accumulation of iron in many organs and tissues including the liver, pancreas, heart, joints, and endocrine organs due to increased absorption of iron in the gastrointestinal tract. Clinical consequences includes cirrhosis of the liver, hepatocellular carcinoma, diabetes, heart failure, arthritis, and hypogonadism. A large number of studies have indicated that hereditary hemochromatosis (HH) is caused by mutations in a gene that encodes a novel member of the major histocompatibility complex class I family initially called HLA-H but now designated as HFE (See, e.g., Feder, J.N., *et al., Nature Genetics*, 13: 339-408, 1996; Beutler, E., *et al., Blood Cells Mol. Dis.*, 22: 187-194, 1996). Most patients with HH are homozygous for the same missense mutation (C282Y) in the gene that encodes HFE. A recent study demonstrated that the C282Y mutant protein is retained in the ER and middle Golgi compartment and is subject to accelerated degradation (Waheed, A., *et al.*, Hereditary hemochromatosis: Effects of C282Y and H63D mutations on association with β2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells, *Proc. Natl. Acad. Sci.*, 94: 12384-12389, 1997). Much of the newly synthesized C282Y mutant HFE protein occurs in a high molecular weight aggregate as is characteristic of misfolded proteins that are retained in the ER or Golgi. The C282Y mutation reduces or prevents association of HFE with β2-microglobulin, which is necessary for normal intracellular transport of HFE and delivery to the cell surface. Thus agents, such as those described herein, that increase or

stimulate the release of misfolded proteins from the ER may be useful in the prevention or treatment of HH by allowing mutant HFE to exit the ER and reach the cell surface.

Gitelman's Syndrome. Gitelman's syndrome is an autosomal recessive disorder characterized by salt wasting and hypokalemia and is caused by mutations in the thiazide sensitive Na-Cl cotransporter (NCC), which is normally expressed in the mammalian kidney at the apical membrane of distal convoluted tubule cells (See, e.g., Simon, D.B., et al., Gitelman's variant of Bartter's syndrome, inherited hypokalemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter, *Nat. Genet.*, 12: 24-30, 1996). In a recent study designed to elucidate the pathogenesis of Gitelman's syndrome, eight mutations corresponding to eight disease-causing mutations found in Gitelman's syndrome patients were introduced into the mouse NCC and studied by functional expression in *Xenopus* oocytes (Kunchaparty, S., *et al.*, Defective processing and expression of thiazide-sensitive Na-Cl cotransporter as a cause of Gitelman's syndrome, *Am J Physiol.*, Oct., 277 (4 Pt 2):F643-9, 1999). Results indicated that a number of the mutations interfere with proper processing and insertion into the plasma membrane. The nearly complete absence of glycosylation argues that the mutant proteins do not exit the ER. The results suggest that at least a subset of Gitelman's mutations, including the most common mutation (G738R), lead to production of proteins that are not glycosylated normally because of misfolding during synthesis. Thus agents, such as those described herein, that increase or stimulate the release of misfolded proteins from the ER may be useful in the prevention or treatment of Gitelman's syndrome by allowing mutant NCC to exit the ER and reach the cell surface.

Cystinuria. Cystinuria is a common inherited disorder characterized by defective transport of cystine and dibasic amino acids through the epithelial cells of the renal tubule and gastrointestinal tract, commonly resulting in the development of cystine calculi (stones) in the kidney. Three types of cystinuria have been described. Mutations in *SLC3A1*, a gene encoding a subunit of the rBAT protein (an amino acid transporter), have been shown to cause Type I cystinuria. In a recent study designed to investigate the pathogenesis of Type I cystinuria, the most common point mutation, M467T and the related mutation M467K were introduced into rBAT and studied by functional expression in *Xenopus* oocytes (Chillarón, J., *et al.*, An Intracellular Trafficking Defect in Type I rBAT Mutants M476T and M467K, *J. Biol. Chem.*, 272(14), 9543-9549, 1997). The study indicated that the mutations interfered with